25°C.

Reaction coil: A column 0.25 μm in inside diameter and 5 m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, add 5 mL of a solution of *o*-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of 2-mercaptoethanol and 2 mL of a solution of lauromacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45°C.

Flow rate of the mobile phase: About 0.6 mL per minute.

Flow rate of the reagent: About 0.5 mL per minute.

System suitability—

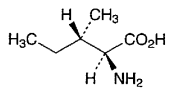
System performance: Dissolve 2 mg of Gentamicin B in 10 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of isepamicin is not more than 3%.

Containers and storage Containers—Tight containers.

L-Isoleucine

L-イソロイシン



$C_6H_{13}NO_2$: 131.17

(2*S*,3*S*)-2-Amino-3-methylpentanoic acid [*73-32-5*]

L-Isoleucine, when dried, contains not less than 98.5% of $C_6H_{13}NO_2$.

Description L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Isoleucine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +39.5 – +41.5° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Isoleucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2, and perform the test using Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Isoleucine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

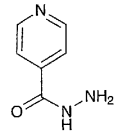
Assay Weigh accurately about 0.13 g of L-Isoleucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.117 mg of $C_6H_{13}NO_2$

Containers and storage Containers—Tight containers.

Isoniazid

イソニアジド



$C_6H_7N_3O$: 137.14
Pyridine-4-carbohydrazide [*54-85-3*]

Isoniazid, when dried, contains not less than 98.5% of $C_6H_7N_3O$.

Description Isoniazid occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

Identification (1) Dissolve about 0.02 g of Isoniazid in water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Isoniazid, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH Dissolve 1.0 g of Isoniazid in 10 mL of freshly boiled and cooled water: the pH of this solution is between 6.5 and 7.5.

Melting point 170 - 173°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of isoniazid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Isoniazid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 0.40 g of Isoniazid according to Method 3, and perform the test using Apparatus B. In this case, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite the ethanol to burn (not more than 5 ppm).

(4) Hydrazine—Dissolve 0.10 g of isoniazid in 5 mL of water, add 0.1 mL of a solution of salicylaldehyde in ethanol (95) (1 in 20), shake immediately, and allow to stand for 5 minutes: no turbidity is produced.

Loss on drying Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.3 g of isoniazid, previously dried, dissolve in 50 mL of acetic acid (100) and 10 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.714 mg of $C_6H_7N_3O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Isoniazid Injection

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Isoniazid Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Injections, with Isoniazid.

Description Isoniazid Injection occurs as a clear, colorless liquid. It has a slight, bitter taste.
pH: 6.5 - 7.5

Identification (1) To a volume of Isoniazid Injection, equivalent to 0.01 g of Isoniazid according to the labeled amount, and add water to make 2 mL. Add 1 mL of silver nitrate-ammonia TS to this solution: a dark turbidity is produced, and a silver mirror is formed on the wall of the test tube with effervescence.

(2) To a volume of Isoniazid Injection, equivalent to 0.1 g of Isoniazid according to the labeled amount, add water to make 4 mL. Add 0.1 g of vanillin and 4 mL of ethanol (95), dissolve by warming moderately, and allow to stand for 3 hours. Collect the precipitated yellow crystals by filtration, and dry at 105°C for 1 hour: the crystals melt between 225°C and 231°C.

Assay To an exactly measured volume of Isoniazid Injection, equivalent to about 0.025 g of isoniazid ($C_6H_7N_3O$), add water to make exactly 250 mL. Pipet 10 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS and water to make exactly 100 mL, and use this solution as the sample solution. Dry isoniazid for assay at 105°C for 2 hours, weigh accurately about 0.025 g of the residue, and dissolve in water to make exactly 250 mL. Pipet 10 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test as directed under the Ultraviolet-visible Spectrophotometry, and determine the absorbances, A_T and A_S, of the sample solution and the standard solution at the wavelength of 267 nm, respectively.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = \text{amount (mg) of isoniazid for assay} \times \frac{A_T}{A_S}$$

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Isoniazid Tablets

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Isoniazid Tablets contain not less than 95% and not more than 105% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Tablets, with Isoniazid.